PROTOCORONOSPORA ON PHORADENDRON FLAVESCENS IN CALIFORNIA

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In the spring of 1928 a new disease of the mistletoe, *Phoradendron flavescens* (Pursh) Nutt. var. *macrophyllum* Engelm. was reported as occurring on mistletoe plants parasitic on willows and poplars growing on the northwest shore of Clear Lake in Lake County, California. Several collections have since been made, but all in this same general locality. The disease has never been noted elsewhere in California, nor has any other record of it been found.

The infection, at first noticeable on the *Phoradendron* leaves as small brown spots 2 to 5 mm. in diameter, later becomes confluent involving the greater part of the leaf (text fig. 1). Stem lesions are smaller, averaging 1 mm. in diameter, but very closely grouped together. In time the infecting organism completely kills its host and fruits abundantly over the surface of the dead leaves and stems. The fruiting bodies are in the form of dark erumpent sori containing masses of spores. The organism causing the disease was at first thought to belong to the genus *Gloeosporium* because of the type of acervuli formed and the manner in which the spore masses oozed from them. An examination of the spore bearing structures, however, showed it to be an undescribed species of the genus *Protocoronospora.*

The genus *Protocoronospora* was first described by Atkinson and Edgerton (Jour. Mycol. 13: 185–186. 1907) as causing a disease of cultivated vetch. These two investigators tentatively placed the new genus close to *Corticium* in the Thelephoraceae because it appeared to produce basidia in a definite hymenium seated on a pseudo-parenchymatous subhymenium. The budding of spores on germination from one or both ends was taken as an additional character indicating basidiomycete relationship. The new genus suggested affinity with *Exobasidium* also in that conidiophores, similar in shape but shorter and more slender than the basidia, were borne in the same hymenium intermingled with the basidia. The conidia resembled basidiospores in shape and size and both were sessile, borne in terminal whorls which were repeatedly replaced.

Wolf, however, in a later and more comprehensive study (Jour. Elisha Mitchell Sci. Soc. 36: 72–84. 1920) of the same organism, *Protocoronospora nigricans,* found characters which definitely removed it from the Thelephoraceae to the Melanconiaceae of the Fungi Imperfecti. His chief basis for the change in position of the genus was the multinucleate condition in all parts of the fungus: nutritive mycelium, stroma, conidio-

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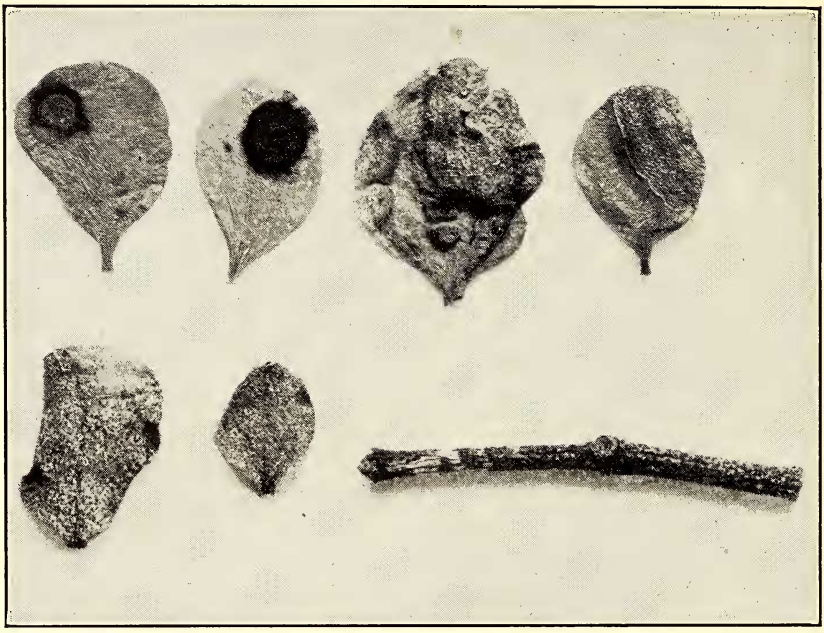

FIG. 1. Leaves and stems of *Phoradendron flavescens* var. *macrophyllum* infected with *Protocoronospora Phoradendri*. The leaves are arranged to show progressive stages in the development of the disease. Collected on *Salix laevigata*, north shore of Clear Lake, Lake County, California. ×0.8.

phores, and conidia. The conidiophores are of varied size, and range from slender, nearly cylindrical to clavate; hence Atkinson's and Edgerton's interpretation of basidia and conidiophores making up the hymenium. A second point of importance lay in the fact that the number of spores borne by each conidiophore is indefinite. This is particularly pertinent since the spores may be replaced, as they mature and fall away, by new ones—a condition not associated with basidiomycetes. Also, in place of being formed on well defined sterigmata, the spores are sessile or nearly so. In the Thelephoraceae, the basidia are binucleate in the young stages, and arise from binucleate cells of the subhymenium. The situation prevailing in *Protocoronospora* is not known in any of the basidiomycetes.

Protocoronospora Phoradendri sp. nov. In maculis brunneis, primum parvis et distinctis, dein confluentibus, in foliis caulibusque; mycelium intracellulare; acervuli amphigeni, subepidermici, erumpentes; setae multum rarae, 30–50 μ longa, 3–4 μ diametro, fuscae; conidiophora clavata ad cylindrata, 11–30 μ longa, 3–9 μ diametro; conidia ab apicibus aut rarius a lateribus conidiophorum abscissa, 1–9 fere 5 aut 6 simul; sterigmata parvula, ob-

scura, non manifesta dum post conidia fundendum; conidia eburnea in mole, recta ubi juvenia, maximum partem falcata ubi matura, 15–26 μ longa, 4.5–6.5 μ diametro; germination a tubulos germinantes producendo.

In brown spots, at first small and distinct, later confluent, on stems and leaves; mycelium intracellular; acervuli amphigenous,

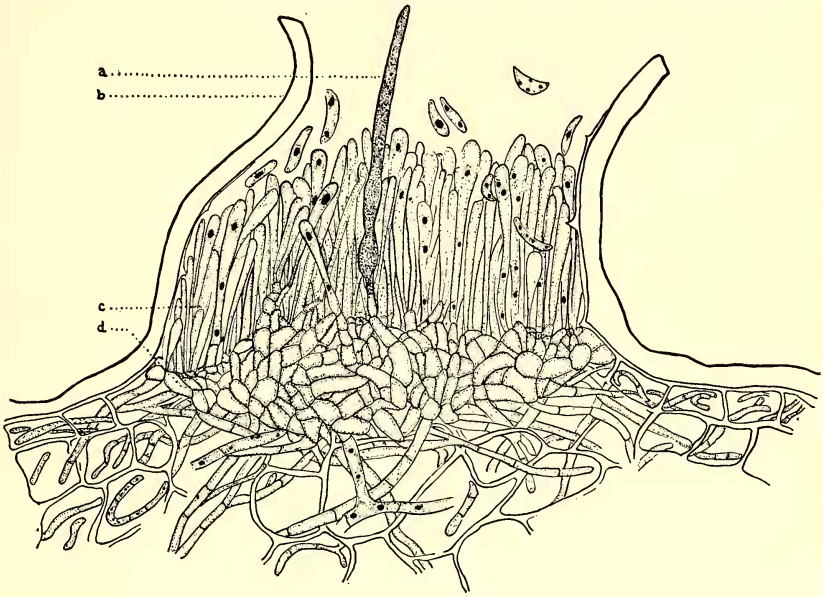

Fig. 2. Young acervulus of *Protocoronospora Phoradendri* on leaf of *Phoradendron flavescens* var. *macrophyllum*: *a* seta, *b* cuticle torn from the epidermis by growth of acervulus, *c* conidiophores, *d* stroma; drawing semi-diagrammatic, × 146.

subepidermal, erumpent; setae exceptionally rare, 30–50 by 3–4 microns, dark brown; conidiophores clavate to cylindrical, 11–30 by 3–9 microns; conidia abstricted from the tips or, more rarely, sides of the conidiophores, 1–9, usually 5 or 6 simultaneously; sterigmata small, obscure, not evident until after shedding of conidia; conidia cream-colored in mass, straight when young, mostly falcate when mature, 15–26 by 4.5–6.5 microns; germination by production of germ tubes only.

Type. On *Phoradendron flavescens* (Pursh) Nutt. var. *macrophyllum* Engelm., north shore of Clear Lake, Lake County, California, March 30, 1934, *Lee Bonar* (Herb. Univ. Calif. no. 615–587).

The morphology of the infection produced on *Phoradendron flavescens* by *Protocoronospora Phoradendri* was studied in detail. Stained permanent preparations of cross sections of leaves in young and more advanced stages of infection were used to work out the structure and development of the acervuli and for study of the nuclear content of hyphal cells in the nutritive mycelium

as well as in the fruiting structures. Examination of this material showed the following: the fungus is an intracellular parasite although it may pass through intercellular spaces as it ramifies through the host. It is most abundant in the epidermal and sub-

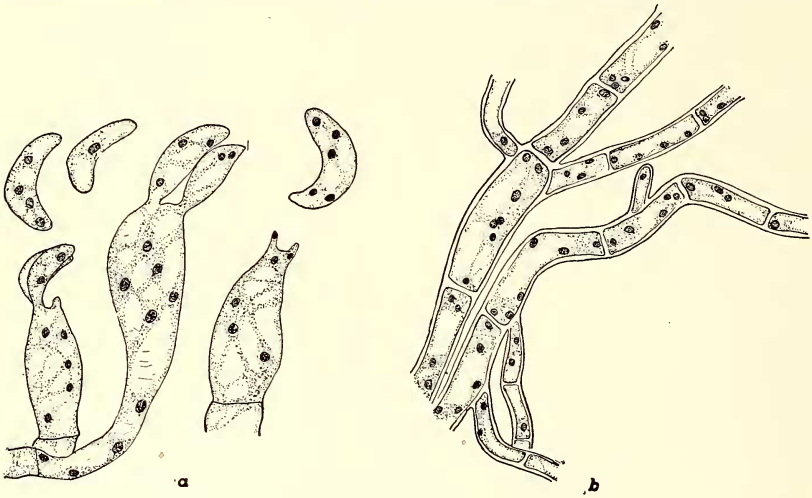

Fig. 3. *Protocoronospora Phoradendri.* *a,* Conidiophores showing sterigmata and spores; from acervulus developed on Leonian's agar; stained to show multinucleate condition, ×298. *b,* Cells of intracellular mycelium showing multinucleate condition; camera lucida drawing, ×194.

epidermal layers and does not invade the vascular tissue. The parasite does not cause distortion in the host cells though the chloroplasts are destroyed with its advance. Hyphal strands in the host cells average 4 to 5 microns in width. Cells with four to six nuclei are common (text fig. 3, b). The mycelium becomes massed at various spots just under the epidermis; there anastomosing strands give rise to a pseudoparenchymatous stroma eventually occupying from two to three layers of host cells. The free ends of stromatic cells form the condiophores. The force exerted by the growth of stroma and conidiophores raises the epidermis, making a rent in the thick cuticle which is finally pushed back, thus forming a border about the whitish pustule of spores extruded from the mouth of the acervulus. With age the averculus becomes darkened and the cuticle around it is sloughed off. Mature acervuli vary in size from 150 to 330 microns, with the average around 250 microns. Sometimes two or more may coalesce. The acervuli form on both surfaces of the leaves.

Stock cultures were started from single spore isolations. A modified Leonian's agar[1] and oatmeal agar proved to be the most

[1]Dihydrogen potassium phosphate ..... 1.2 gr.
Magnesium sulphate ..... .5 gr.
Agar ..... 20. gr.
Glucose ..... 6. gr.
Malt extract ..... 6. gr.
Distilled water ..... 1,000 cc.

suitable media both for mycelial growth and for sporulation. Colonies in culture are characterized by a flat, "wet" type of growth. Often almost as soon as macroscopic colonies are evident on the agar, dark cushion-like centers are developed. These are bordered by a white margin of actively growing hyphae. Zonation is a conspicuous feature in older colonies of this fungus.

Two to three days after conidia have been planted on plates of Leonian's agar, fresh conidia develop as lateral outgrowths from hyphal cells as well as on conidiophores. Spores form as outgrowths of the cells and are abstricted as they enlarge and become curved. Mature spores measuring up to 30 by 8 microns may be found massed about the mycelium and conidiophores. Usually within a week black acervuli of irregular shape develop on the young colonies. Conidiophores and conidia form in great numbers in these structures and ooze forth from the mouths.

The conidiophores in culture show variation in shape although all are of the same general pattern, cylindrical to clavate. They are borne on the tips of the hyphae and often are not differentiated to any extent from other cells in the filament. The conidia are borne in whorls at the tips and occasionally along the upper sides of the conidiophores. The number borne at a time varies from one to eight or nine. They appear to be sessile until after they have fallen away when little sterigmata-like pegs may now and then be seen projecting from the condiophores. Presumably these little projections grow into new spores which, in turn, when abstricted, leave sterigmata at the base. The conidia are hyaline, granular, thin-walled, multinucleate, sometimes containing guttulae. They cling together in glutinous, cream-colored masses.

Infection experiments were carried on with three species of plants: *Vicia sativa* to which *Protocoronospora nigricans* is extremely destructive, *Phoradendron flavescens* var. *macrophyllum* and *Phoradendron villosum*. Plants were inoculated as follows: (1) by sponging the leaves and stems with a heavy spore suspension and (2) by wounding the tissues with a needle dipped in the spore suspension. Neither *Vicia sativa* nor *Phoradendron villosum* proved susceptible to attack. In the case of *Phoradendron flavescens* var. *macrophyllum*, infection in the form of blackening from 1 to 2 mm. about the wounds showed within eight days. Within forty-three days immature acervuli appeared. The plants which were sponged with spore suspension were not affected. In leaves inoculated through wounding, the incidence of infection was approximately 30 per cent.

For purpose of contrast, specific characters of *Protocoronospora nigricans* and *Protocoronospora Phoradendri* are assembled in the following table. Material used for the study of *Protocoronospora nigricans* was furnished by Dr. Wolf.

TABLE 1. Comparison of *Protocoronospora nigricans* and *Protocoronospora Phoradendri.*

Protocoronospora nigricans	*Protocoronospora Phoradendri*
Leaf lesions numerous, small, dark brown to black, spots av. 1 mm., or in streaks 1–5 mm. long.	*Leaf lesions* lighter brown, large, indefinite in shape, becoming confluent over most of leaf.
Stem lesions, black streaks 3 mm. to 3 cm. or more.	*Stem lesions* small, 1 mm. or less, crowded, coalescent.
Acervuli 55–220 microns diam., av. less than 100 microns, single or clustered, subepidermal, erumpent.	*Acervuli* 165–330 microns diam., single or clustered, more conspicuously erumpent.
Setae dark brown, tapering, 50–95 by 6–9 microns, abundant on leaves and stems, none on pods; 1–20, av. 6–8 per acervulus.	*Setae* dark brown, tapering, bulbous base, 30–50 long by 3–4 microns wide above base; very rare on leaf, lacking on stems; 1–10, av. 1–2 per acervulus.
Conidiophores clavate to cylindrical.	*Conidophores* clavate to cylindrical.
Conidia straight to slightly curved, continuous, 11–20 (av. 17) by 2.5–3.5 microns, hyaline, pale pink in mass.	*Conidia* decidedly falcate at maturity, continuous, 15–26 (av. 20–24) by 4.5–6.5 microns, hyaline, creamy white in mass.
Germination of conidia by budding and by germ tubes.	*Germination* of conidia by germ tubes; no true budding.
Hosts: species of *Vicia.*	*Host: Phoradendron flavescens* var. *macrophyllum.*

These differences are considered of sufficient importance to distinguish the fungus parasitic on mistletoe as a separate species.

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